

## REMARKS

### Status of the Claims

Claims 27-52 are pending in the present application. Claims 1-26 were previously canceled. Claims 34-42 and 46-51 are withdrawn as directed to a non-elected invention. Claims 27, 30-31, and 43-44 are amended. Claim 52 is new. Support for amended claim 27 is found throughout the application as originally filed including on page 8, lines 20-23 and Examples 1 and 2. Claims 30-31 are amended for clarity and/or to correct antecedent basis. Claims 43-44 are amended for clarity according to the Examiner's suggestions.

New claim 52 describes that the measurement is carried out by visual observation of a change in color of the colored compound (substrate). This feature is on pages 11 and 12, bridging paragraph, of the originally filed application, which states as follows:

The present inventors further invented pigments whose color is changed by the enzyme reaction by protease. That is, the present inventors discovered that when a protease acts on a colored compound which is a pigment having at least one amino group, in which an amino acid(s) and/or oligopeptide(s) is(are) bound to one or more of the at least one amino group through an amide bond(s), the amide bond(s) is(are) cleaved, that results in color change of the compound. The term "color change" herein means that both colors of the compound before and after the enzyme reaction can be visually seen, and the color change is discernible by visual observation. Color change may be more simply observed than fluorescence which requires excitation light, and even a slight change is more readily discernible than coloring (colorless compound is colored), so that observation of color change is advantageous.

In view of the foregoing, no new matter is entered by way of this amendment. Reconsideration is respectfully requested.

### Amendments to the Specification

#### *Sequence Listing*

The specification is amended to insert a sequence listing. Enclosed herewith in full compliance with 37 C.F.R. §§1.821-1.825 is a Sequence Listing to be inserted into the specification as indicated above. The Sequence Listing in no way introduces new matter into the

specification. Also submitted herewith in full compliance with 37 C.F.R. §§1.821-1.825 is an electronic CRF copy of the Sequence Listing. The electronic CRF copy of the Sequence Listing, file “2011-04-10 0760-0356PUS1\_ST25.txt”, is identical to the paper copy, except that it lacks formatting. The enclosed paper copy and the electronic CRF copy of the Sequence Listing do not include new matter.

The specification is also amended to properly identify each disclosed sequence from the description with a corresponding sequence identification number (SEQ ID NO). Pursuant to 37 C.F.R. §1.821(a), only amino acid sequences with four or more specifically defined amino acids were included in the Sequence Listing. No new matter is introduced by these amendments.

*Further Amendments to the Specification*

The specification is also amended to include priority data and to correct the structure of cresyl violet. Applicants submit that an ordinary artisan would have recognized that the structure of cresyl violet in the originally filed application is incorrect. Further, since the structure of cresyl violet was known in the art at the time of the invention, an ordinary artisan would have recognized the correct structure.

In addition, the Brief Description of the Drawings is amended to correct an obvious typographical error. Specifically, the description of Fig. 11 and Fig. 12 refer to adhesive sheets, which are correctly depicted in Fig. 10, rather than Fig. 11.

No new matter is entered by way of the above-described amendments. Entry is respectfully requested.

**Statement of the Substance of the Interview**

Applicants and Applicants’ representative thank the Examiner for extending the courtesy of an interview on March 22, 2011. The substance of the interview is substantially as described in the Examiner interview summary, which issued on March 28, 2011. Briefly, Applicants’ representative requested a copy of the Sequence Compliance Notice, which the Examiner subsequently provided.

### **Objections to the Specification**

#### *Priority Data*

The Examiner objects to the specification because the priority data is not described on line 1 of the originally filed application, *see Office Action*, page 3, item 8. As noted above, the specification is amended to add the priority data. Accordingly, Applicants believe the objection is overcome and respectfully request withdrawal.

#### *Structure of Cresyl Violet*

The Examiner further objects to the specification because he alleges that the structure of cresyl violet is inconsistent with that known in the art, *see Office Action*, page 3, item 9, and pages 1-2 of Examiner's search, enclosed.

As noted above, the specification is amended to correct the structure of cresyl violet. Accordingly, Applicants believe the objection is overcome and respectfully request withdrawal.

#### *Sequence Listing*

The Examiner objects to the specification because sequences are described in the specification, but no sequence listing has been provided, *see Office Action*, page 3, item 10. As noted above, the specification is amended to incorporate a sequence listing. Accordingly, Applicants believe the objection is overcome and respectfully request withdrawal.

#### *Brief Description of the Figures*

The Examiner further objects to the specification because the Brief Descriptions of Figures 11 and 12 are allegedly unclear, *see Office Action*, page 3, item 10. Applicants have amended the Brief Descriptions of the Figures to indicate that the adhesive sheets are depicted in Fig. 10. Accordingly, Applicants believe the objection is overcome and respectfully request withdrawal.

### **Objections to the Claims**

Claims 43 and 44 are objected to as allegedly lacking clarity, *see Office Action*, pages 3-4, item 11.

The claims are amended as suggested by the Examiner. Accordingly, Applicants believe the objection is overcome and respectfully request withdrawal.

**Issue under 35 U.S.C. § 112, First Paragraph**

Claims 27-33 and 43-45 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement, *see Office Action*, pages 4-7, item 13.

This rejection is respectfully traversed.

According to the Examiner, the specification fails to support that allergens in the test material may be detected or quantified without pretreatment. The Examiner interprets the term “pretreatment” to mean any manipulation of the sample including suspension in phosphate buffer or incubation.

As amended, claim 27 specifies “contacting said test material with a substrate of said protease without pretreatment of the test material, wherein the pretreatment of the test material is selected from the group consisting of extraction, concentration, and purification of the allergens in the test material.” Applicants submit that the present application supports this subject matter *see* page 8, lines 20-23 and Examples 1 and 2 in the instant application. Examples 1 and 2 do not require extraction, concentration, and purification of the allergens prior to detection or quantitation using the claimed methods.

In view of the foregoing, Applicants believe that the amended claims comply with the enablement requirement. Accordingly, reconsideration and withdrawal of this rejection are respectfully requested.

**Issues under 35 U.S.C. § 102(b)**

Claims 27-28, 30, 32, and 33 are rejected under 35 U.S.C. § 102(b) as allegedly anticipated by GB 2351560 to Pirzad (“Pirzad”), *see Office Action*, pages 7-8, item 15.

This rejection is respectfully traversed.

According to the Examiner, Pirzad teaches all of the elements of the instant claims including pretreatment.

As amended, claim 27 is directed to a method for measuring an environmental biological allergen(s), which comprises measuring said biological allergen(s) by measuring protease activity of said allergen(s), wherein said allergen(s) is in a test material(s); contacting said test

material with a substrate of said protease without pretreatment of the test material, wherein the pretreatment of the test material is selected from the group consisting of extraction, concentration, and purification of the allergens in the test material; and correlating the presence or absence or quantity of protease activity with the presence or absence or quantity of allergens.

Applicants submit that Pirzad's method requires that the allergens (proteases or by-products of mites for example) be extracted or concentrated from the test sample before detection, *see, for example*, page 3, lines 2-24, and page 2, lines 23-24 of Pirzad. Accordingly, Pirzad does not encompass the amended claims, which specify "contacting said test material with a substrate of said protease without pretreatment of the test material, wherein the pretreatment of the test material selected from the group consisting of extraction, concentration, and purification of the allergens in the test material."

More particularly, as expressly stated on page 13, lines 17 to 19 of Pirzad, the collected dust is washed with 0.1M NaHCO<sub>3</sub>, 0.5 M NaCl (pH8.3). As is well-known to an ordinary artisan, NaHCO<sub>3</sub> is an alkali (this is also evident from the pH of 8.3). Therefore, although the term "wash" is used, this is nothing other than an alkali extraction, *see also* step 50 (PROVIDE DUST SAMPLE") in Figs. 1 and 4 of Pirzad.

Applicants note that in some Examples of the present application, alkali treatment is used as a pretreatment. These Examples support claim 1 as-filed in Japan. That is, the original claim 1 filed in Japan encompasses test materials with a pretreatment. The instant amended claims encompass test materials without pretreatment selected from the group consisting of extraction, concentration and purification.

In view of the foregoing, the amended claims are not anticipated by Pirzad. Accordingly, withdrawal of the rejection is respectfully requested.

#### **Issues under 35 U.S.C. § 103(a)**

Claims 29 and 43-44 are rejected under 35 U.S.C. § 103 as allegedly obvious over Pirzad and U.S. Patent No. 5,667,979 to Berrens, *see Office Action*, pages 9-13, item 18.

Claims 28 and 31 are also rejected under 35 U.S.C. § 103 as allegedly obvious over Pirzad and Berrens in further view of Ino *et al.*, 1989, *Archives of Allergy and Applied Immunology*, 89:321-326 ("Ino"), *see Office Action*, pages 11-13, item 19.

Claim 45 is rejected under 35 U.S.C. § 103 as allegedly obvious over Pirzad and Berrens in further view U.S. Patent No. 6,235,493 to Bissell *et al.* (“Bissell”), *see Office Action*, pages 13-14, item 20.

These rejections are respectfully traversed.

Claims 28, 29, 31, 43-45 and 52

*Basis for the Rejection*

According to the Examiner, Pirzad teaches all of the elements of the instant claims except for the features in dependent claims 28, 29, 31, and 43-45. Nevertheless, the Examiner believes that Berrens, Ino, and Bessell remedy these deficiencies.

*The cited references do not teach or suggest all of the claimed features.*

As noted above, independent claim 27 is amended to specify “contacting said test material with a substrate of said protease without pretreatment of the test material, wherein the pretreatment of the test material is selected from the group consisting of extraction, concentration, and purification of the allergens in the test material.” Applicants submit that none of the cited references, either alone or in any combination, teach or suggest this feature. This feature is incorporated into dependent claims 28-33, 43-45, and 52. Accordingly, the cited references fail to teach or suggest all of the elements of dependent claims 28-33, 43-45, and 52.

*The claimed methods result in unexpected effects.*

Applicants further submit that an ordinary artisan could not have reasonably expected that the protease activity of environmental biological allergens could have been measured without pretreatment of the test material as described in the present claims. In this regard, as stated on page 8, lines 20 to 27 of the present application states:

Further, surprisingly, as will be described concretely in Examples below, it was discovered by the present inventors that the protease activity of environmental biological allergens may be measured without any pretreatment such as extraction, concentration or purification of the allergens. Thus, in a preferred mode of the present invention, the environmental biological allergen(s) is(are) subjected to the measurement as it(they) is(are) or after merely

being dissolved or suspended in water or in a buffer, without any pretreatment such as extraction or purification, so that the method is extremely simple.

Accordingly, at the time of the invention, an ordinary artisan could not have reasonably expected that protease activity of environmental biological allergens may be measured without any test material pretreatment, such as extraction, concentration and purification of the allergens.

Claims 43-45 and 52

As noted above, dependent claims 43-45 and 52 are not rendered obvious by the cited references at least because the claims incorporate the feature of measuring protease without pretreatment of the test material. Applicants further note, however, that the features further specified in claim 43 are not taught or suggested in Pirzad and Berrens.

Claim 43 specifies that the substrate is a colored compound comprising at least one amino group, at least one amino acid and/or at least one oligopeptide, wherein the at least one amino group, the at least one amino acid and/or the at least one oligopeptide is bound to a pigment via an amide bond.

Applicants submit that the combination of references does not describe a colored compound. Although Berrens discloses the use of a chromogenic substrate, this is not the "colored compound" recited in claim 43. More particularly, "chromogenic" means, in its definition, "to generate color." Berrens exemplifies the "chromogenic substrate", H-D-Isoleucyl-L-proyl-L-arginine-*p*-nitroanilide-di HCl, ("I.P.A."). Although this substrate is "chromogenic", the substrate *per se* is colorless.

*p*-nitroaniline, however, which is to be produced by the cleavage of the tripeptide moiety in I.P.A. is yellow. Therefore, *via* a protease reaction, the yellow compound is produced by the decomposition of the tripeptide moiety by the action of the protease. Since the amount of the generated yellow compound is proportional to the enzyme activity, by measuring the coloring by, for example, measuring the absorbance, the amount of protease activity can be measured. Thus, Berrens utilizes "coloring" or "color generation", and not "color change."

In contrast, in the method of claim 43, "color change", not "color generation" is utilized. The definition of "color change", is described in the specification on page 11, lines 23-26, which states:

The term "color change" herein means that both colors of the compound before and after the enzyme reaction can be visually seen, and the color change is discernible by visual observation.

The advantage of utilizing the "color change" over "color generation" is also described in the specification on page 11, lines 26 to page 12, line 2, which states:

Color change may be more simply observed than fluorescence which requires excitation light, and even a slight change is more readily discernible than coloring (colorless compound is colored), so that observation of color change is advantageous.

Applicants further submit that although the pigments *per se* used in the claimed method, such as, cresyl violet, Safranin O and methylene violet 3RAX were known in the art at the time of the invention, the compounds in which such a pigment is bound to the peptide, as well as use thereof for the measurement of protease activity, was not known at the time of the invention.

In view of the foregoing, the claims are not rendered obvious by the cited references. Accordingly, reconsideration and withdrawal of this rejection are respectfully requested.

### CONCLUSION

In view of the above-described claim amendments and remarks, Applicants believe the present application is in condition for allowance.

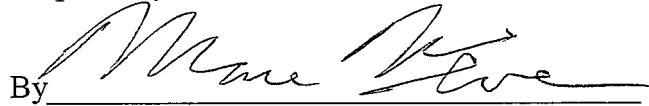
Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Linda T. Parker, Ph.D., Registration No.46,046 at the telephone number of the undersigned below to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Director is hereby authorized to charge any fees required during the pendency of the above-identified application or credit any overpayment to Deposit Account No. 02-2448.

AUG 17 2011

Dated: \_\_\_\_\_

Respectfully submitted,

By 

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Attachment(s): Sequence Listing